IN VITRO HYPOGLYCEMIC AND RADICAL SCAVENGING ACTIVITIES OF CERTAIN MEDICINAL PLANTS

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ABSTRACT

The purpose of this study is to investigate in vitro hypoglycemic and free radical scavenging activities of some medicinal plants including Ficus glomerata (FG), Pandanus amaryllifolia (PaA), Artocarpus altilis (AA), Gomphrena celosioides (GC) and Gynostemma pentaphyllum (GP). Alpha-amylase inhibitory assay was examined by dinitrosalicylic acid reaction. Glucose uptake assay was investigated by LO-2 cell model. DPPH and $ABTS^+$ scavenging assays were performed by spectrophotometry. Cell viability was determined by MTT method. It was found that the extracts including FG, PaA, AA, GC and GP were able to inhibit alpha-amylase activity up to 38.4 ± 4.2%, 47.8 ± 4.3%, 49.3 ± 3.5%, 40.1 ± 4.4% and 38.5 ± 3.8%, respectively. Moreover, glucose adsorption and glucose uptake capacity of these extracts were evidenced. In addition, free radical scavenging activity of these extracts was indicated in a range of 30.6-54.5% for DPPH radical and 31.8-51.1% for $ABTS^+$ radical. Especially, these extracts exhibited no cytotoxicity effect on human hepatic LO-2 cells and human gastric BGC-823 cells at the concentration of 100 µg/ml. The results indicated that A. altilis leaves were effective in inhibiting alpha-amylase activity, increasing glucose adsorption and glucose uptake and scavenging free radicals. Therefore, it could be suggested to be a promising hypoglycemic agent for managing type 2 diabetes.

Keywords: *Alpha-amylase, anti-diabetes, antioxidant, medicinal plants, glucose uptake substitutes; ischemia treatment.*

INTRODUCTION

Diabetes is one of the most frequent non-communicable lifestyle-related diseases in the world. According to World Health Organization projections, around 300 million people will be affected by diabetes by the year 2025 (1). Especially, a major metabolic disorder prevalence of diabetes mellitus is increasing daily. It is characterized by the relative or absolute deficiency of insulin secretion and/or insulin action that causes glucose intolerance and impairs carbohydrate, lipid and protein metabolisms (2, 3). Patients affected by diabetes mellitus develop different diabetes mellitus-related complications such as retinopathy, nephropathy and peripheral neuropathy (4). Currently, the oral anti-diabetic drugs, such as sulfonylureas, biguanides, α-glucosidase inhibitors, thiazolidinediones and dipeptidyl peptidase-4 inhibitors are the approved medications for type 2 diabetes mellitus (5). However, these drugs are less effective in long-term regulation of the glycemic level and exhibit many undesirable side effects. Therefore, the discovery of safer and more effective anti-diabetic agents is still necessary.

Plants produce a huge array of natural products with high diversity of structures (6, 7). Hundreds of active compounds have been identified in plants including terpenoids, alkaloids, flavonoids and phenolic compounds (8). However, 90% useful natural lead compounds are still waiting for the discovery in the future (9). Since ancient times, numerous plants have been used as herbal medicines to treat various diseases due to their secondary metabolites (10, 11). Up to now, the plant-based medicines are still a common tendency for primary health care as these are affordable and safer to use (12). Notably, herbal medicines have played an important role in the prevention and treatment of type 2 diabetes via prophylactic and therapeutic management (13, 14). Prophylactic action of herbal medicines may be attributed to healthy organs and their cellular tissue, especially beta cells of pancreas and hepatic tissue. Meanwhile, therapeutic action of herbal medicines may be due to curative action on affected tissue of pancreas, liver and diabetes-related organs (15). Hence, medicinal plants are considered as a bright future in the therapy and management of diabetes. Especially, medicinal plants such as *Ficus glomerata, Pandanus amaryllifolia, Artocarpus altilis, Gomphrena celosioides and Gynostemma pentaphyllum* have been known as useful herbs for the treatment of various diseases. *F. glomerata* is an evergreen tree of Moraceae family widely distributed throughout warmer parts of Asia, Africa, America, and Australia. It has been used for the treatment of biliary disorders, jaundice, dysentery, diabetes, diarrhoea and inflammatory conditions (16). *P. amaryllifolia* is a tropical plant from Pandanaceae family which is widely used in South and Southeast Asia for cooking. *P. amaryllifolius* leaves have a number of local medicinal uses in reducing fever, relieving indigestion and flatulence, and decreasing postprandial blood sugar (17, 18). *A. altilis* belongs to Moraceae family and is grown throughout Southeast Asia, Pacific Ocean islands, Senegal, Ghana, Liberia and India (19). The medicinal values of *A. altilis* have been known due to the treatment of tongue thrush, skin infections, sciatica, diarrhoea, low blood pressure, asthma and diabetes (20). *G. celoisiodes* is a sprawling herb which belongs to Amaranthaceae family and spreads throughout the whole tropical world, especially Brazil, Paraguay, Uruguay and Argentina (21). It has been recognized in different traditional systems of medicines for the treatment of various diseases such oliguria, heat and empacho, hypertension, cough, diabetes, kidney problems, jaundice, high cholesterol, bronchial asthma and fever (22). *G. pentaphyllum* is a perennial creeping herb of Cucurbitaceae family. It disperses throughout India, Nepal, Bangladesh, Sri Lanka, Laos, Myanmar, China, Korea and Japan. *G. pentaphyllum* is applied as herbal medicine for the treatment of haematuria, oedema and pain of the pharynx, heat and oedema of the neck, tumours and trauma, haematuria, hyperlipidaemia, palpitation and shortness of breath, chest congestion, dizziness, headache, forgetfulness, tinnitus and spontaneous perspiration (23). Notably, these plants have received a lot of attention due to lowering blood glucose in type 2 diabetes. However, the scientific reports regarding anti-diabetic activity of these medicinal plants are still limited. Accordingly, these five medicinal plants have been subjected and screened for their potential anti-diabetic effect via inhibiting alpha-amylase activity and scavenging free radicals.

MATERIALS AND METHODS

Materials

Leaves of *Ficus glomerata*, *Pandanus amaryllifolia* and *Artocarpus altilis* were collected from Tay Ninh province, Vietnam (April 2018), while all parts of *Gomphrena celosioides* and *Gynostemma pentaphyllum* were bought from the local market in Vietnam (District 5, Ho Chi Minh city). Acarbose and Metformin were purchased from the pharmacy store at district 7, Ho Chi Minh city, Vietnam. Alpha-amylase from *Bacillus licheniformis* (A4582) was purchased from Sigma-Aldrich (USA). Solvent was purchased from Xilong (China). All other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Extraction

Materials were air-dried under shade and powdered by a grinder. The powder was soaked with ethanol under the extract conditions: ratio (1/4, w/v), time (4 h) and temperature (60 ^{0}C). The ethanol extracts of these plants were kept at 4 ^{0}C for further investigation. The concentration of 100 µg/ml of the extracts was used for all assays.

Alpha-amylase inhibitory assay

The determination of alpha-amylase inhibition was carried out by dinitrosalicylic acid (DNS) method (24). Briefly, 1ml of the extract (100 µg/ml) was pre-incubated with 1 ml of alpha-amylase (diluted for 10000 in 20 mM sodium phosphate buffer, pH 6.9) for 30 min and 1 ml (1% w/v) of starch solution was then added into the mixture. The mixture was further incubated at 37 ^{0}C for 10 min. The reaction was then

stopped by adding 1 ml of DNS reagent (12.0 g of sodium potassium tartrate tetrahydrate in 8 mL of 2 M NaOH and 96 mM 3,5-dinitrosalicylic acid solution), followed by the heating of contents in a boiling water bath for 5 min. The control (C) was prepared without plant extracts and the blank (B) was without the alpha-amylase enzyme. The absorbance was measured at 540 nm. Acarbose (100 µg/ml) was used as reference. The inhibition of alpha-amylase in percentage was calculated by the following equation (OD_B is the absorbance of blank, while OD_C is the absorbance of control):

$$\text{Inhibition (\%)} = \frac{(OD_C - OD_B) - (OD_{sample} - OD_B)}{(OD_C - OD_B)} \text{ x } 100\%$$

Glucose adsorption capacity assay

Glucose adsorption capacity of the extracts was determined *in vitro* (25). The extracts (1%) were added to 25 ml of glucose solution (50 mM). The mixture was well mixed and incubated at 37 ^{0}C for 6 h, centrifuged at 4000g for 20 min and the glucose content in the supernatant was determined. The bound glucose was calculated using the following formula (G1 is the glucose concentration of original solution; G2 is the glucose concentration after 6 h incubation):

The glucose adsorption (mM glucose/g extract) =
[(G1 – G2)*Volume of solution]/Weight of sample

Glucose uptake capacity assay

Human hepatic LO-2 cells were cultured in a humidified atmosphere containing 5% CO_2 at 37 ^{0}C using Dulbecco's modified eagle medium (DMEM) supplemented with 5% heat-inactivated fetal bovine serum (FBS), 10 mM HEPES buffer, 100 U/ml of penicillin G, and 100 mg/ml of streptomycin. The glucose uptake into LO-2 cells was determined *in vitro* (26). Briefly, the cells ($1x10^4$ cells/ml) were incubated with the extracts (100 µg/ml) or metformin (20 µg/ml) for 48 h. The spent culture medium was then removed and replaced with 50 µl incubation buffer (0.1% BSA and 8 mM glucose) and further incubated for 3 h at 37 ^{0}C. Afterward, glucose concentration in supernatant was measured using Contour™ Plus Meter (Ascensia Diabetes Care, Switzerland). The percentage of glucose uptake was calculated as a percentage compared to control C (The untreated cell group). The percentage of glucose uptake was calculated by the following formula (T is glucose concentration in supernatant of the treated cell group, while C is glucose concentration in supernatant of the untreated cell group):

Glucose uptake (%) = [(8 – T)/(8 – C)]*100

1,1-Diphenyl-2-picryl-hydrazyl assay

The antioxidant activity of the extracts was determined by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay (27). Briefly, 100 µl of the extracts (100 µg/ml) was mixed with 100 µl of DPPH (3 mM) solution and incubated in the dark at room temperature for 30 min. The absorbance of the mixture was then measured at 490 nm by using Genova Nano (Jenway, UK). Vitamin C (20 µg/ml) was used as a reference. DPPH scavenging ability was determined by the following formula (OD: absorbance or optical density):

DPPH scavenging ability (%) =
$[(OD_{control} - OD_{sample})/OD_{control}]$ x 100%

2,2-Azinobis-3-Ethyl benzothiazoline-6-sulfonic acid assay

The antioxidant activity of the extract was also determined by 2,2-azinobis-3-ethyl benzothiazoline-6-sulfonic acid assay (27). Briefly, the photometric assay was conducted on 0.9 ml of $ABTS^+$ solution and 0.1 ml of the extracts (100 µg/ml), mixing for 45 sec. Measurement was taken immediately at 734 nm after 15 min incubation. Vitamin C (20 µg/ml) was used as a reference. The $ABTS^+$ scavenging ability was determined by the following formula (OD: absorbance or optical density):

ABTS scavenging ability (%) =
$[(OD_{control} - OD_{sample})/OD_{control}]$ x 100%

Cell viability assay

The percentage of viable cells (LO-2 and BGC-823) was determined after 24h treatment with investigated agents using MTT assay (28). Briefly, the cells ($1x10^5$ cells/ml) were incubated with 100 µg/ml of the extracts for 24 h. The medium was removed, and the cells were incubated with a solution of 1 mg/ml MTT for 4 h. Finally, supernatant was removed, and DMSO was added to solubilize the formed formazan salt. Amount of formazan salt was determined by measuring absorbance at 540 nm using a microplate reader. The cell viability was calculated as a percentage compared to blank.

Statistical analysis

Data were analysed using a one-way analysis of variance test of the statistical package for social sciences (SPSS). The statistical differences among groups were assessed using Duncan tests. The differences were considered significant at $p < 0.05$.

RESULTS

Alpha-amylase inhibitory activity of the extracts

The extracts of various medicinal plants including FG, PaA, AA, GC and GP were investigated for their capability against alpha-amylase activity. The results showed that these extracts exhibited inhibitory activity on alpha-amylase at the concentration of 100 µg/ml (Figure 1). Among them, AA and PaA extracts possessed the highest inhibition on alpha-

amylase activity. The inhibitory effects of AA and PaA extracts were observed up to 49.3 ± 3.5% and 47.8 ± 4.3%, respectively, followed by GC - 40.1 ± 4.4%, GP - 38.5 ± 3.8% and FG - 38.4 ± 4.2%. Meanwhile, the inhibitory activity of acarbose (59 ± 5.3 %) was higher than that of these extracts.

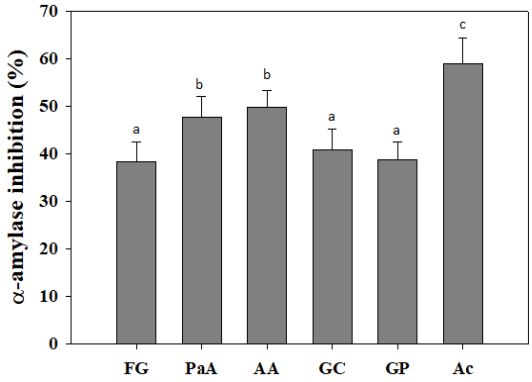


Figure 1. The alpha-amylase inhibitory activity of the extracts. Each determination was made in three independent experiments, and the data are shown as means ± SD. Different letters a–c indicate significant difference among groups ($p < 0.05$). *Ficus glomerata* (FG), *Pandanus amaryllifolia* (PaA), *Artocarpus altilis* (AA), *Gomphrena celosioides* (GC), *Gynostemma pentaphyllum* (GP), and Acarbose (Ac).

Glucose adsorption capacity of the extracts

In this assay, glucose adsorption capacity of FG, PaA, AA, GC and GP extracts at the concentration of 1% (w/v) were investigated *in vitro* (Figure 2). It was observed that GC extract exerted the highest glucose adsorption capacity at the value of 1.8 ± 0.16 mM glucose/g extract, followed by AA (1.7 ± 0.18), PaA (1.2 ± 0.15), GP (1.1 ± 0.11) and FG (1.0 ± 0.13) mM glucose/g extract.

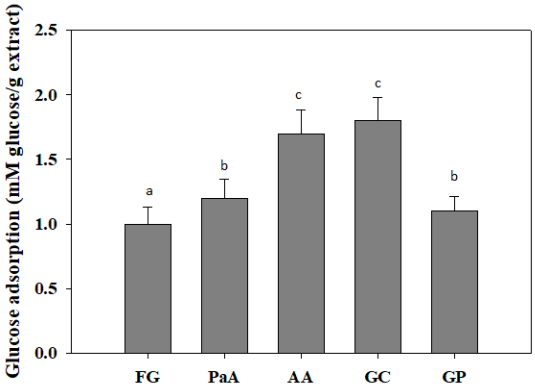


Figure 2. Glucose adsorption capacity of the extracts at glucose concentrations of 50 mM. Each determination was made in three independent experiments and the data are shown as means ± SD. Different letters a–c indicate significant difference among groups ($p < 0.05$). *Ficus glomerata* (FG), *Pandanus amaryllifolia* (PaA), *Artocarpus altilis* (AA), *Gomphrena celosioides* (GC) and *Gynostemma pentaphyllum* (GP).

The glucose uptake capacity of the extracts

In order to investigate whether the extracts are able to stimulate glucose uptake, hepatic LO-2 cells were pre-treated with different extracts before incubated with glucose solution. The percentage of glucose uptake was indicated by measuring the rest of glucose level in the cell culture supernatant. The results showed that PaA and AA extracts possessed the highest stimulation of glucose uptake as compared to the control group. The glucose uptake levels of PaA and AA were up to 143 ± 11.3% and 142 ± 9.1%, respectively, followed by GC - 128 ± 1.0%, FG - 115 ± 1.0% and GP - 115 ± 9.1%. Meanwhile, metformin-treated cells significantly increased glucose uptake up to 197 ± 11.3% at the concentration of 20 µg/ml.

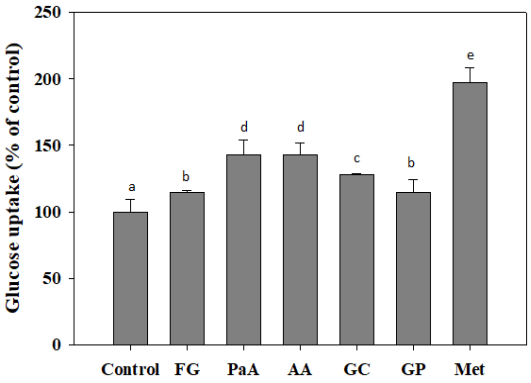


Figure 3. Glucose uptake capacity of the extracts on human hepatic LO-2 cells. Each determination was made in three independent experiments and the data are shown as means ± SD. Different letters a–e indicate significant difference among groups ($p < 0.05$). Control is the untreated cell group. *Ficus glomerata* (FG), *Pandanus amaryllifolia* (PaA), *Artocarpus altilis* (AA), *Gomphrena celosioides* (GC), and *Gynostemma pentaphyllum* (GP), and metformin (Met).

Free radical scavenging activity of the extracts

Antioxidant activity of the extracts was investigated by measuring DPPH and ABTS+ radical scavenging ability. In Figure 4A, high DPPH scavenging activity was exposed by AA (54.5 ± 2.5)% and FG (41.2 ± 2.2)%, followed by GP (37.5 ± 4.8)%, PaA (34.9 ± 3.5)%, and GC (30.6 ± 5.1)% at the concentration of 100 µg/ml. Likewise, these extracts also exhibited ABTS+ scavenging activity up to 52.4 ± 4.6% for FG, 51.1 ± 5.2% for AA, 36.6 ± 4.7% for GC, 36.3 ± 2.9% for PaA, and 31.8 ± 3.5% for GP (Figure 4B). Besides, vitamin C was indicated as a powerful scavenger of DPPH (81.8 ± 4.3%) and ABTS+ (98 ± 3.8%) radicals.

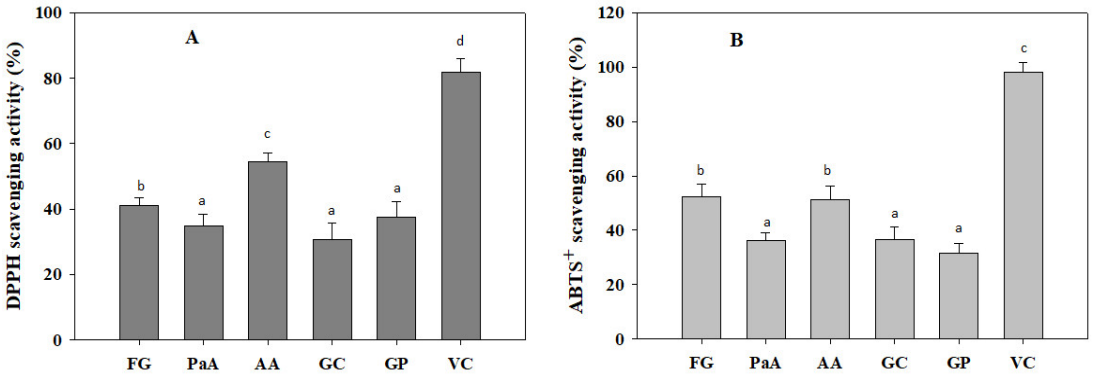


Figure 4. DPPH (A) and $ABTS^{+}$ (B) scavenging activity of the extracts.
Each determination was made in three independent experiments and the data are shown as means ± SD.
Different letters a–d indicate significant difference among groups ($p < 0.05$).
Ficus glomerata (FG), *Pandanus amaryllifolia* (PaA), *Artocarpus altilis* (AA),
Gomphrena celosioides (GC), and *Gynostemma pentaphyllum* (GP), and Vitamin C (VC).

Effect of the extracts on cell viability

The effect of FG, PaA, AA, GC, and GP extracts on cell viability of human hepatic LO-2 cells and human gastric BGC-823 cells was investigated *in vitro* (Figure 5). The cell viability was shown in a range of 86 – 99% for LO-2 cells and 83 – 95% for BGC-823 cells as compared with the blank group (Absence of extract). This indicates that FG, PaA, AA, GC, and GP extracts did not cause any cytotoxic effect on human hepatic cells and human gastric cells at the concentration of 100 µg/ml.

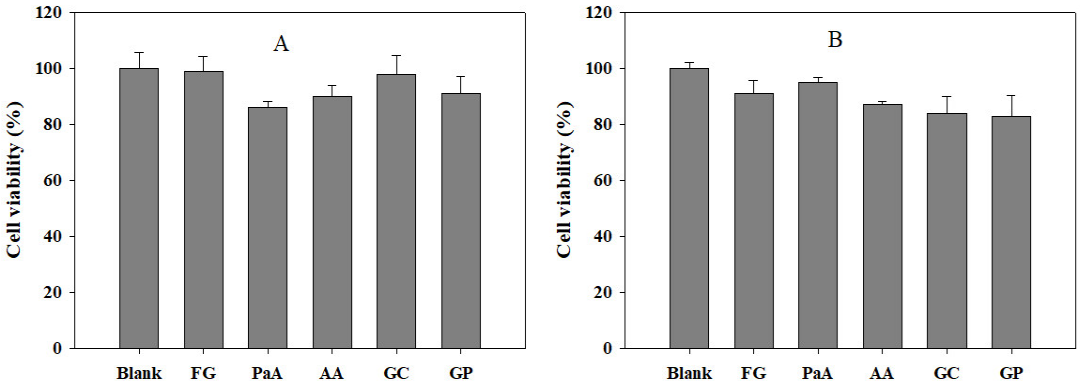


Figure 5. The effect of the extracts on cell viability of human hepatic LO-2 cells (A) and human gastric BGC-823 cells (B) using MTT assay. The cells were treated with the extracts for 24 h before adding MTT reagent. The results were expressed as compared with blank group.
Each determination was made in three independent experiments and the data are shown as means ± SD.

DISCUSSION

Alpha-amylase plays an important role in hydrolysis of carbohydrates to small fragments of sugars in intestinal mucosa. Normally, the excess level of sugar will be converted to energy sources such as glycogen. In diabetic patients, the excess activity of alpha-amylase enzyme results in hyperglycaemia due to insulin deficiency or insulin resistance (29). In this sense, the inhibition of alpha-amylase activity can reduce postprandial hyperglycemia and prevent the risk of diabetes development (30, 31). In this study, the extracts of AA, PaA, GC, GP and FG were found to possess inhibitory activity against alpha-amylase. Notably, the inhibitory activity of AA and PaA extracts on alpha-amylase was more effective than that of *Momordica charantia* (IC_{50} = 0.267 ± 0.024 mg/ml) (32). Currently, acarbose, miglito, and voglibose are used as common anti-diabetic drugs for down-regulation of carbohydrate digestion enzymes such as alpha-amylase, sucrose,

maltase, and alpha-glucosidase (33). It was evidenced that mice treated with acarbose could slow the breakdown of sucrose and starch (34, 35). Hence, the inhibitory activity of these extracts, especially AA and PaA, on alpha-amylase may contribute to the diminution of hyperglycaemia in type 2 diabetes patients.

Indeed, the hypoglycemic effect of medicinal plants not only associates to the inhibitory activity of carbohydrate digestion enzymes, but also relates to their adsorption capacity of glucose. Notably, various hypoglycemic medicinal plants such as ginseng, bitter melon, fenugreek, banaba, *Gymnema sylvestre* and *Coptis chinensis* have been known due to their glucose adsorption capacity (36). Herein, FG, PaA, AA, GC, and GP extracts were also evidenced as potential adsorptive agents of glucose. The glucose adsorption capacity of these extracts may be attributed by their phytoconstituents such as insoluble and soluble constituents and fibers (37). As the result, these extracts, especially GC extract, were suggested to be able to decrease transport across of glucose into intestinal lumen and reduce the postprandial hyperglycemia.

It was reported that insulin resistance or insulin deficiency causes decrease in glucose uptake and increase in endogenic hepatic glucose production in diabetic patients (38). Thus, bioactive agents possessing glucose uptake capacity play an important role in hypoglycemia via stimulating glucose uptake into skeletal muscle, adipose tissue and liver (39). Notably, FG, PaA, AA, GC and GP extracts were determined to stimulate glucose uptake into hepatic LO-2 cells significantly. The glucose uptake capacity of these extracts, especially PaA and AA, may be associated to the recruitment of glucose transporters from intracellular pool to plasma membrane of the cells, thus stimulating glucose uptake in its target tissues and attenuating the hyperglycemia in type 2 diabetes (40).

Free radicals cause oxidation of cell components and molecules such as lipids, proteins, and DNA (41). Notably, free radical activity has been previously implicated in the development of diabetic vascular complications in diabetes mellitus (42). Long term complication of diabetes mellitus is associated with various oxidative reactions, free radical generation and oxidative stress (43). Therefore, antioxidants play a central role in neutralization of free radicals and prevention of the pathogenesis as well as complications of diabetes mellitus (44). Interestingly, antioxidant activity of FG, PaA, AA, GC and GP extracts was found due to scavenging DPPH and $ABTS^+$ radicals. It was evidenced that the transgenic antioxidant enzyme expression or antioxidant compounds have the capacity to prevent the development of experimental diabetic retinopathy, nephropathy, neuropathy and cardiomyopathy (42). Zatalia et al. (44) recently listed the beneficial effects of antioxidant agents for the treatment of diabetes and its complications in animals and humans. These experimental and human studies have suggested nutritional values in the prevention of diabetic complications. Thus, the potential antioxidant property of these extracts, especially FG and AA, may contribute to delay the development of free radicals-related diabetes complications.

Obviously, medicinal plants have long been used for maintaining human health and continued to be a valuable source of pharmaceuticals up to now (45). Besides great significance in therapeutic treatments, they also possess cytotoxic potential due to producing various cytotoxic substances for defence purposes (46). Thus, an assessment of their cytotoxic potential is necessary to ensure relatively safe use of medicinal plants. In the present study, MTT assay has revealed that the ethanol extract of these medicinal plants did not cause any cytotoxicity on hepatic LO-2 cells and gastric BGC-823 cells at the tested concentrations (100 µg/ml). Moreover, the results indicate that BGC-283 cell viability was lower after AA, GC and GP treatment as compared to the blank. According Jamil et al. (47), AA extract reduced cell viability of HeLa cervical cancer cells up to 44% at 100 µg/ml. Similarly, GP extract lowered cell viability of A549 human lung epithelial cells up to 21% at 100 µg/ml (48). Therefore, a further study is needed due to cytotoxic effect of these extracts using an *in vivo* model to achieve the adequate knowledge regarding safe use of these medicinal plants.

CONCLUSIONS

Herbal medicines have traditionally been used for the prevention and treatment of various diseases up to now. The pharmacological researches using *in vitro* as well as *in vivo* models have evidenced numerous health-beneficial effects of herbal medicines. In this study, different medicinal plants including *F. glomerata, P. amaryllifolia, A. altilis*, *G. celosioides and G. pentaphyllum* have been analysed as a promising hypoglycemic agents due to inhibiting starch digestive enzyme, possessing glucose adsorption and glucose uptake capacity and scavenging free radicals. Especially, *A. altilis* was observed to be effective in all assays, indicating its promising therapeutic role in the management of hyperglycaemia in type 2 diabetes. However, further studies related to safety and efficacy of *A. altilis* also need to be evaluated for future development of health-beneficial products.

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